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Examining the Conspecific Audience Effect on 22-kHz Ultrasonic Distress Vocalizations in Rats

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QUESTIONS

- When rats are stressed with a series of mild foot shocks, will they produce different patterns of distress vocalizations in the presence of conspecifics versus when they are alone?
- Will these patterns of distress vocalizations change as the number of conspecifics changes?

INTRODUCTION

- Rats produce a 22 kHz distress vocalization during times of stress and anxiety, and these vocalizations are a reliable behavioral measure of discomfort. [1,5,6,7]
- Distress calls have been observed for a number of species, ranging from common chickens (gallus gallus) to trumpeter swans and even bats.[2,3,4]
- Previous research by Blanchard, et al. suggests that rats vocalize differently based on social factors, such as group size, and isolation status, such as being along in a burrow versus being with others rats. [1,7]
- Blanchard, et al. found a significant sex difference in the number of vocalizations and the types of vocalizations when examining anti-predator distress calls. [1]
- We will examine the effect of varying the audience size (0, 1, or 3 rats) on the number of distress vocalizations produced during a period of aversive stimulation (mild foot shocks).
- We hypothesize that the rats with three audience members will vocalize more often than the rats with zero audience members.
  - We will examine the vocalization behavior of both male and female rats to determine if there is a differential vocalization pattern.

METHODS

- Seventy-Eight Long-Evans rats served as subjects in this experiment:
  - •39 Males and 39 Females
- The two sexes were tested under each of three conditions:
  - •No Audience (n = 26)
  - •Audience Size = 1 rat (n = 26)
  - •Audience Size = 3 rats (n = 26)
- The subject rats were placed in a transparent operant conditioning chamber with the audience rats in an adjacent transparent Plexiglas cage. The subject rats were given a mild foot shock at the end of the first, second, third, fourth and fifth minutes in the cage.
  - •The audience rats never served as subjects in the experiment in order to keep them naïve regarding the cause of the distress signals.
- The microphone capsule of a heterodyne ultrasound detector (Ultrasound Advice U30) was mounted inside the operant conditioning chamber. The detector was tuned to 22 kHz and its output was routed to the microphone input of a video camera (Sony Digital 8) allowing us to record both the subjects' behavior and 22 kHz vocalizations onto videotape for later analysis.
- Vocalizations were counted using software specifically written for this analysis, which greatly assisted the researchers in examining the total number of 22 kHz calls produced for the duration of the experiment.
- Differences in number of vocalizations per minute were analyzed using a one-way Analysis of Variance (ANOVA) to compare the effects of sex differences and audience size on 22 kHz vocalization behavior.

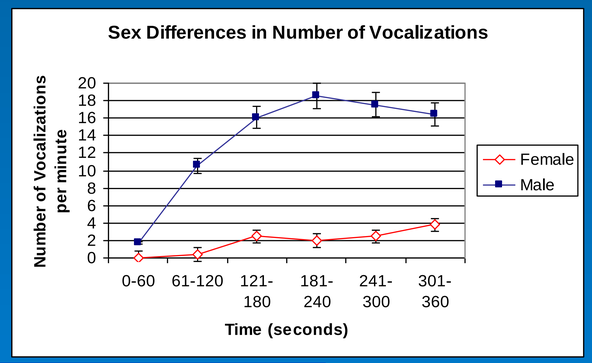

Figure 1

The number of vocalizations of Male rats (n=39) and Female rats (n=39). Each point is represented as mean ± Standard Error of the Mean. ANOVA revealed a significant effect of sex and time at each point except for the 0-60 second time period. $F(1,77)=44.306$, $P<0.001$ as well as a significant interaction. $F(5,389)=22.798$, $P<0.001$.

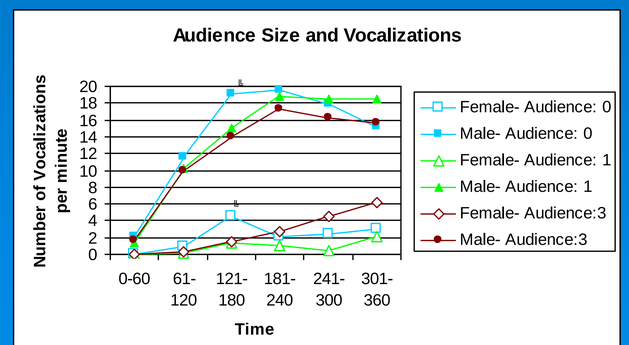

Figure 2

The number of vocalizations per minute plotted with sex and audience effect breakdown. Female groups (N=39; n=13 per audience group) show a significantly lower number of vocalizations than male rats. A point of interest, however, is the marked groups Female Audience 0 (n=13) and Male Audience 0 (n=13) which show distinctive peaking patterns at the 121-180 second time period (indicated by ‖).

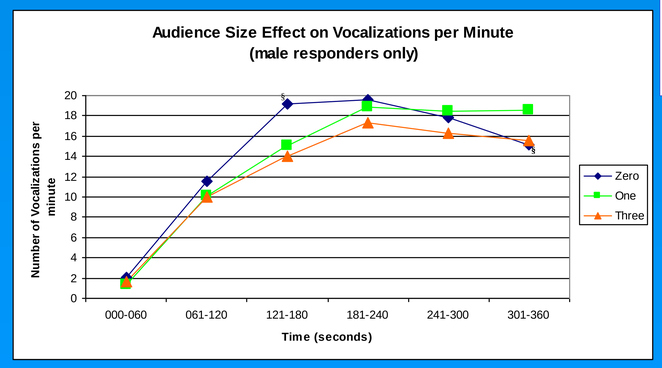

Figure 3

Due to a skewing effect from non-responding rats (rats that did not vocalize up through the 121-181 time period) we graphed just the responding rats to get a clearer picture of the trends in vocalization. For the zero audience group (n=12) there is a clear peak in vocalizations after the second foot shock (at 120 seconds) but a quick drop-off of vocalizations while the one audience group (n=10) and three audience group (n=11) maintain consistent vocalization patterns. The two points (Zero audience, 121-180 seconds & Zero Audience, 301-360; marked §) show a significant decline (One-tailed T-Test $T(15)=7.220$, $P<.001$)

CONCLUSIONS & DISCUSSION

In general, there were differential patterns of vocalization.

As hypothesized,

- There was a significant difference between the vocalization behavior of males and females.
- An interesting pattern emerged with regard to audience effect on vocalization:
  - •The "no audience" group shows a quick increase in the number of vocalizations followed by a quick drop in number of vocalizations.
  - •The one- and three-member audience groups rise more slowly than the no audience group, but plateau, with the one-member audience group showing slightly more vocalizations.
  - •The "no audience" group showed a significant decline between the two points 121-180 seconds & 301-360 seconds shown by a One-Tailed T Test. See Fig. 3
  - •These results suggest that further research is needed to more closely examine the differences between audience size and vocalization behavior.
- Examining a longer period of time (i.e. 12 minutes instead of 6 minutes) might show the significant difference in vocalization more clearly. [5,6]
- Furthermore, the results also support testing of 22 kHz vocalization behavior in males only.
- To examine whether vocalization duration changes with audience size, the vocalizations will be analyzes using frequency analysis.

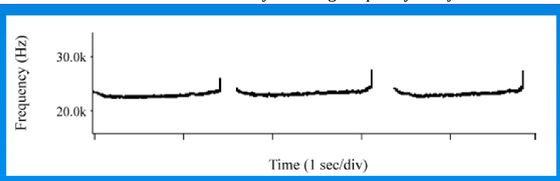

Figure 4

The frequency of 22 kHz distress calls are plotted out on a time scale. Using a personal computer to plot these calls out and analyze the total call duration and call content (by means of Fourier analysis) researchers would be able to get a clearer picture of what these calls mean, and have a greater spectrum of variables to analyze. *(Figure reproduced from Inagaki et al. [5])*

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